REMARKS

Applicant notes with appreciation the establishment of a continued prosecution application and the withdrawal of the prior objections to Claims 3, 171, and 196-199. Applicant further notes with appreciation the indication of allowability of Claims 86-96, 173, 176, and 194-195.

Claims 1 and 3-213 are pending in the application. Applicant respectfully acknowledges the withdrawal of Claims 1, 85, 97-106, 108-117, 120-121, 123-167, 170, 177-188, and 200-213. Claims 3-84, 107, 118, 119, 122, 168, 169, 171, 172, 174, 175, 189-193, and 196-199 are at issue in the case.

The application has been reviewed for minor typographical errors. Claims 190-193 and 196-199 have been amended according to the Examiner's suggestion. Applicant respectfully submits that those claims as amended comply with the requirements of 35 U.S.C. §112, 2nd paragraph.

Furthermore, Claim 4 has been amended to independent form. Applicant accordingly respectfully submits that Claims 4-84, 107, 118-119, 122, 168, 169, 172, 174, 175, and 189 are in proper condition for allowance.

The Action maintains the judicially created double patenting rejection of Claims 3, 171, 190-192, and 196-199 over claims 19-23, 25-29, 31, and 34 of U.S. Patent No. 5,932,479. Applicant intends to address this rejection with a suitable terminal disclaimer once these claims have been found otherwise patentable.

Applicant further acknowledges the provisional obviousness-type double patenting rejection of Claims 3, 171, and 190-192 over Claims 119-120, 124, 132, 140-142, 153-157, 166-167, and 188 of copending Application No. 08/972,901. Applicant intends to address this

rejection with a suitable terminal disclaimer if and when Application No. 08/972,901 matures into a patent, and if those claims are determined to be otherwise allowable.

Applicant respectfully submits that Claims 3, 171, 190-192, and 196-199 are sufficiently enabled by the disclosure, as the disclosure sufficiently informs those skilled in the art how to make and use the claimed invention. The prevailing standard for determining whether the specification meets the enablement requirement is whether the experimentation needed to practice the invention is undue or unreasonable. *See* Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916); *In re* Wands, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the active scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied.

According to that standard for determining enablement, Applicant respectfully submits that the phrase "said chloroplast (or flanking) sequences being conserved in all higher plants" in Claims 190-192 and Claims 196-199 is enabled by the disclosure. The Specification at pages 27-28 provides extensive instructions for identifying appropriate intergenic spacer sequences. Specifically, the method for identifying appropriate spacer regions involves isolating plastid genomic DNA, hybridizing with a radioactive labeled probe of a known spacer, and detecting and isolating plastid sequences which exhibit the desired degree of homology with the probe. Alternatively, the BLAST program may be used to identify highly conserved regions in plastid genomes. This is particularly true in light of the publication of the complete DNA sequence and genomic maps of at least fourteen different plant species. The following complete chloroplast genome sequences are already available in Genbank:

Marchantia polymorpha 121,024 kbp
Nicotiana tabacum 155,844 kbp
Oryza sativa 134,525 kbp
Epifagus virginiana 70,028 kbp
Pinus thunbergii 119,707 kbp

Zea mays 140,387 kbp
Arabidopsis thaliana 154,478 kbp
Triticum aestivum 134,540 kbp
Euglena gracilis 143,172 kbp
Cyanophora paradoxa 135,599 kbp
Odontella sinensis 119,704 kbp
Porphyra purpurea 191,028 kbp
Chlorella vulgatis 150,613 kbp
Mesostigma viride 118,360 kbp

Thus one could, if so inclined, simply search for polycistronic spacer regions from one plant species in other species to determine whether that particular region is conserved. This method would not be difficult, and one skilled in the art would certainly find highly conserved regions in this manner. In addition, numerous incomplete chloroplast genome sequences are also available in the Genbank. With rapid advances in DNA sequencing technology, almost all other needed chloroplast genome sequences are expected to be deposited in the Genbank shortly. Therefore, the BLAST search suggested in the specification to identify transcriptionally active spacer regions should be a routine procedure for one skilled in the art.

The thus identified flanking sequences are then subcloned into a plasmid for construction of the universal chloroplast integration and expression vector, as demonstrated in Example 1 at page 41 of the Specification. Moreover, working examples using a universal border sequence comprising the spacer region between the trnI and trnA genes are provided. That working example is representative of a broad class of flanking sequences that can be used according to the claimed invention. As these are standard techniques in the art, one skilled in the art would thus be able to make and use the claimed invention without undue experimentation.

Furthermore, the specification describes in detail how to make UV chloroplast vectors using DNA sequences that are available on Genbank using tobacco spacer 2 as an example, and shows how the BLAST search should be done. The specification provides examples of searching for conserved spacer regions from a complete genome sequence (tobacco, maize, rice, Epifagus) or incomplete sequence (soybean, sunflower, pea, spinach, Oenothera, Alnus, Cuscuta)

available in Genbank (figure 4 A-G). Figures 5, 6, 7 A-D and 8, give a detailed description of how restriction fragments are cut and pasted together, after identification of desired spacer regions.

On page 27, the section on identification of intergenic spacer sequences, also describes how to identify conserved spacer regions with required homology by performing Southern hybridization and subsequent restriction digestion to cut out restriction fragments from unknown chloroplast genomes to make UV chloroplast vectors. For such unknown chloroplast sequences, no sequence information is necessary from Genbank. Different conditions for Southern hybridization to isolate regions with different homologies are provided in the specification (page 27). Figures 5, 6, 7 A-D and 8, also give a detailed description of how restriction fragments are cut and pasted together, after identification of desired spacer regions. Applicant accordingly respectfully submits that the solicited claims are fully enabled by the Specification as originally filed.

Applicant further respectfully submits that integration of heterologous DNA into transcriptionally active regions of the chloroplast genome of higher plants in Claims 190-191 and 196-197 is fully enabled by the disclosure of the Specification. Unlike the prior art, such as Zoubenko, whereby integration occurs at a transcriptionally inactive region wherein two promoters direct the transcription of genes in opposite directions away from the silent region of the chromosome, the invention integrates a cassette containing a transcription terminator into a transcriptionally active region of the chloroplast genome. Promoters control the expression of the one or more inserted genes, as explained in the Specification at page 6. In light of the disclosure, Applicant respectfully submits that one skilled in the art would thus be able to make and use the claimed invention without undue experimentation. Accordingly, Applicant respectfully requests withdrawal of the §112, first paragraph rejections to Claim 3, 171, 190-192, and 196-199.

Turning to the prior art, Applicant respectfully submits that the Zoubenko reference does not anticipate Claim 192. Claim 192 is drawn to a universal integration and expression vector comprising flanking sequences conserved among higher plants and complementary to the corresponding chloroplast sequences of the target plant. In sharp contrast, the Zoubenko article teaches insertion of the heterologous DNA in the trnV-rps 12/7 intergenic region. As explained at page 3822, column 1 of Zoubenko, that region of the chloroplast genome is unique to the tobacco plastid genome. As Zoubenko does not teach flanking sequences that are conserved among higher plants, Applicant respectfully requests withdrawal of the rejection to Claim 192 on the grounds of that reference.

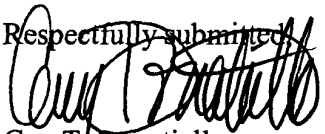
Applicant also respectfully submits that the Staub 1993 reference does not anticipate Claim 192. That claim requires that the flanking sequences be derived from a plant species different from the target plant. In sharp contrast, Staub 1993 teaches that the flanking regions are derived from tobacco plastid DNA. See "Materials and Methods" section on page 605. Additionally, only tobacco plants were transformed with the plastid transformation vector. Accordingly, Staub 1993 cannot anticipate Claim 192.

Applicant further respectfully submits that Claims 3, 171, and 190-192 as amended are patentably distinct over Staub 1995 in light of Sidorov. Staub 1995 teaches the use of flanking regions derived from the rbcL/accD intergenic region of the tobacco plastid genome. Claims 3, 171, and 190-192 have been amended to specify that the integration is into a polycistronic region of the chloroplast genome. A polycistron comprises a series of genes under the control of a common promoter. Thus the individual genes have no individual promoters. Rather, a single promoter initiates transcription of all genes in the operon. Since the genes are under the control of a single promoter, they are all transcribed at the same time, to the same number of transcripts. Of course, a gene with a separate promoter could be introduced in the operon, but it would not change the fact that this is a polycistronic region of the genome, as it naturally occurs.

In Staub, the rbcL and accD genes each have their own promoters, as stated in the legend to Figure 1 on p 846, column 1 of the reference. The rbcL promoter can read through the rbcL gene into the subsequent (otherwise) spacer region, but the read through is undependable (about 30%). More to the point, since the accD gene has its own promoter, this region (between the rbcL gene and the accD gene) is not part of a polycistron or operon. The recitation of Sidorov fails to remedy this deficiency. Sidorov only discloses that the flanking sequences of the tobacco vector are homologous to the potato plastid genome and thus homologous recombination was anticipated. Sidorov also suggests, however, that the low transformation efficiency may be due to poor homologous recombination. Thus, even assuming that Sidorov supports the assertion that it was inherent that the vector of Staub 1995 could be inserted into a chloroplast genome of a plant other than tobacco, there is no support in Sidorov for the claimed insertion of the flanking sequences into a polycistronic region of the plant genome. Nor is there support for the claimed conservation of the flanking sequences in *all* higher plants. Staub 1995 does not remedy this deficiency. Staub 1995 similarly fails to teach or suggest the use of flanking sequences conserved among all higher plants. The gene order of the rbcL and accD genes disclosed in Staub is not conserved among all plant chloroplast genomes (see Maier et al. (1995) J. Mol. Biol. 251, 614-628, a copy of which is submitted herewith, which disproves such an assertion). Applicant therefore requests withdrawal of the rejection of Claims 3, 171, and 190-192 on the basis of Staub 1995 in view of Sidorov.

In light of the foregoing, Applicant respectfully submits that the solicited claims are in proper form for allowance. Early notification to that effect is respectfully requested.

Respectfully submitted,



Guy T. Donatiello
Reg. No. 33,167
Attorney for Applicant

GTD/FG:jr
(215)563-1810

Marked-Up Version Showing Changes Made to the Claims

4. (Twice Amended) ~~The vector of claim 3,~~ A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, chloroplast DNA sequences which originate from a plant species different from the target plant, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent to undergo homologous recombination with said complementary sequences, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated by said homologous recombination of the flanking sequences with the complementary sequences in the target chloroplast genome, wherein said stable integration is not directed into a transcriptionally inactive region of the chloroplast genome, said vector comprising a heterologous nucleotide sequence coding for a selectable phenotype wherein the flanking sequences comprise, each one a portion of the intergenic spacer 2 region between the $tRNA^{Ile}$ and the $tRNA^{Ala}$ genes of the chloroplast genome of a higher plant, which plant is the same as or different from the target higher plant, whereby double homologous recombination with the conserved spacer 2 region in the target plant chloroplast genome is facilitated.

190. (Twice Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from

the 3' ends of the coding ~~sequences~~ sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, chloroplast DNA sequences which originate from a plant species different from the target plant, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent of ~~undergoing~~ to undergo homologous recombination with said complementary sequences, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated by said homologous recombination of the flanking sequences with the complementary sequences in the target chloroplast genome, and wherein said stable integration is ~~not~~ directed into a transcriptionally ~~in~~active polycistronic spacer region of the chloroplast genome.

191. (Twice Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding ~~sequences~~ sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent ~~of undergoing~~to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence ~~into a transcriptionally active region of the chloroplast genome of the target plant~~ is facilitated through homologous

recombination of the flanking sequences with the homologous sequences in the target chloroplast genome into a transcriptionally active polycistronic spacer region of the chloroplast genome of the target plant.

192. (Twice Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding ~~sequences~~ sequence including a transcription termination region to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent ~~of undergoing~~to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence ~~into the chloroplast genome of the target plant~~ is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome into a transcriptionally active polycistronic spacer region of the chloroplast genome of the target plant.

193. (Twice Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding ~~sequences~~ sequence to provide expression of the coding sequence in the

chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking chloroplast DNA sequences each one a portion of a synthetic spacer 2 region between the $tRNA^{Ile}$ and $tRNA^{Ala}$ genes, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent ~~of undergoing~~to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in a transcriptionally active polycistronic spacer region of the target chloroplast genome.

196. (Twice Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding ~~sequences~~ sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent ~~of undergoing~~to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome~~,~~ of different plant species,

whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome, and wherein said stable integration is ~~not~~ directed into a transcriptionally ~~in~~active polycistronic spacer region of the chloroplast genome.

197. (Twice Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding ~~sequences~~ sequence to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent ~~of undergoing~~to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence ~~into a transcriptionally active region of the chloroplast genome of the target plant~~ is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome into a transcriptionally active polycistronic spacer region of the chloroplast genome of the target plant.

198. (Twice Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding ~~sequences~~ sequence to provide expression of the coding sequence including a transcription termination region in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent ~~of undergoing~~to undergo homologous recombination with said complementary sequences of the target plant and which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in a transcriptionally active polycistronic spacer region of the target chloroplast genome.

199. (Twice Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding ~~sequences~~ sequence to provide expression of the

coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent ~~of undergoing~~to undergo homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence in a transcriptionally active polycistronic region of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome and the vector does not include a transposon.